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The Effect of Ozone on Human Cellular and Humoral Immunity: Characterization of T and B Lymphocytes by Rosette Formation

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Eight healthy male subjects were exposed to $784 \mu\text{g}/\text{m}^3$ (0.4 ppm) of ozone for 4 hours in an environmentally controlled exposure chamber. Peripheral blood samples were taken before and after air and ozone exposures and again at 72 hours and 2 weeks. These blood samples were examined for the percentages of rosette-forming T and B lymphocytes. No statistically significant depressions in T-lymphocyte rosette formation with sheep erythrocytes were found following exposure to air or ozone. The ability of B lymphocytes to form rosettes with sensitized human erythrocytes was depressed following ozone exposure. These results suggest that surface receptors and/or the cell membranes of peripheral blood B lymphocytes may be temporarily altered following short-term exposure to ozone.

INTRODUCTION

Ambient ozone concentrations as high as $1176 \mu\text{g}/\text{m}^3$ (0.6 ppm) have been measured in southern California (Environmental Protection Agency, 1974). The toxic effect of ozone (O_3) in animals and humans has been well documented (Stokinger and Coffin, 1968; U.S. Department of Health, Education and Welfare, 1970). Limited data on the immunological response to O_3 are available. Matsumura (1970a-c) reported that O_3 induced and enhanced allergic reactions in exposed guinea pigs when they were subsequently exposed to antigen aerosols.

Zelac *et al.* (1971) reported an increase in the number of chromosomal aberrations in the lymphocytes of Chinese hamsters following exposure to $392 \mu\text{g}/\text{m}^3$ (0.2 ppm) of O_3 for 5 hours. *In vitro* suppression in the mitogen-induced proliferation of T lymphocytes has been noted in 20 humans exposed to $784 \mu\text{g}/\text{m}^3$ (0.4 ppm) of O_3 for 4 hours (Peterson, personal communication).

Rosette formation is an *in vitro* method of identifying lymphocytes, without prior immunization, in which antigenic red blood cells react with surface membrane sites. Different antigenic red blood cells are used to distinguish T from B cells. The microscopic pattern observed resembles a "rosette." Identification by rosette formation has been utilized in the characterization of various disease or stress states (Aiuti *et al.*, 1975). The purpose of our investigation was to determine the potential effect of a short-term exposure to O_3 on lymphocytes in the cellular and humoral immune systems.

MATERIALS AND METHODS

Subject selection and exposure. Eight male volunteers, ages 19–26, were studied after informed consent was obtained. All subjects completed a comprehensive medical questionnaire and were given a physical examination by a physician. The subjects were healthy nonsmokers with no history of respiratory disease. Exposures were performed in an environmentally controlled chamber designed by Strong *et al.* (1975). Volunteers were first exposed to air, and then after a 3-day interval they were exposed to $784 \mu\text{g}/\text{m}^3$ (0.4 ppm) $\pm$ 10% of O_3 for one 4-hour period. Ten to fifteen milliliters of venous blood were drawn for each subject before and immediately after air and O_3 exposures, and again at 72 hours and 2 weeks following the exposure to O_3 . These samples were analyzed for the percentage of rosette-forming cells (RFC).

Lymphocyte separation. Lymphocytes were separated from the whole blood sample by a modification of the procedure recommended for use with lymphocyte separation medium (Litton Bionetics, Kensington, Md.). Equal portions of blood and saline were mixed and layered above LSM (Ficoll-Hypaque). The mixture was centrifuged and lymphocytes were harvested from the interface (90–95% lymphocytes). The cells were washed with Hanks' balanced salt solution (Grand Island Biological Co., Grand Is., N.Y.), enumerated, and diluted with HBSS to yield a suspension containing 2×10^6 lymphocytes/ml.

Lymphocyte characterization. T and B lymphocytes were distinguished by specific cell-type surface membrane characteristics using the procedures described by Mendes *et al.* (1973). T lymphocytes were identified by their ability to form rosettes with sheep erythrocytes (E). B lymphocytes were identified by their ability to form rosettes with human erythrocytes (HE) sensitized with rabbit antiserum (A) and mouse complement (C). Two hundred lymphocytes were counted. A lymphocyte with three or more red cells attached was counted as a rosette. The percentage of RFC was calculated for duplicate samples and the average percentage for the pair was recorded.

Statistical analysis. The T and B cell data were analyzed using a one-factor analysis of variance for repeated measures. Three hypotheses were tested: (1) no difference in pre- O_3 and post- O_3 means; (2) no difference in pre- O_3 and 72-hour post- O_3 means; and (3) no difference in pre- O_3 and 2-week post- O_3 means.

RESULTS

Figure 1 is an illustration of an E rosette that was used to identify a T lymphocyte. B-Cell rosettes are similar.

No statistically significant difference was found in the mean percentage of E rosettes ($P = 0.188$) or HEAC rosettes ($P = 0.692$) formed before and after air exposure.

The mean number of E rosettes formed in six of the eight subjects tested appeared to decrease following ozone exposure (Table 1), however this difference when compared to pre- O_3 exposure was not statistically significant: post O_3 ($P = 0.171$); 72 hours ($P = 0.135$); and after 2 weeks ($P = 0.889$). The analysis of variance is shown in Table 2.

The mean percentage of HEAC rosettes formed by all eight subjects was reduced immediately following ozone exposure and this reduction was still present

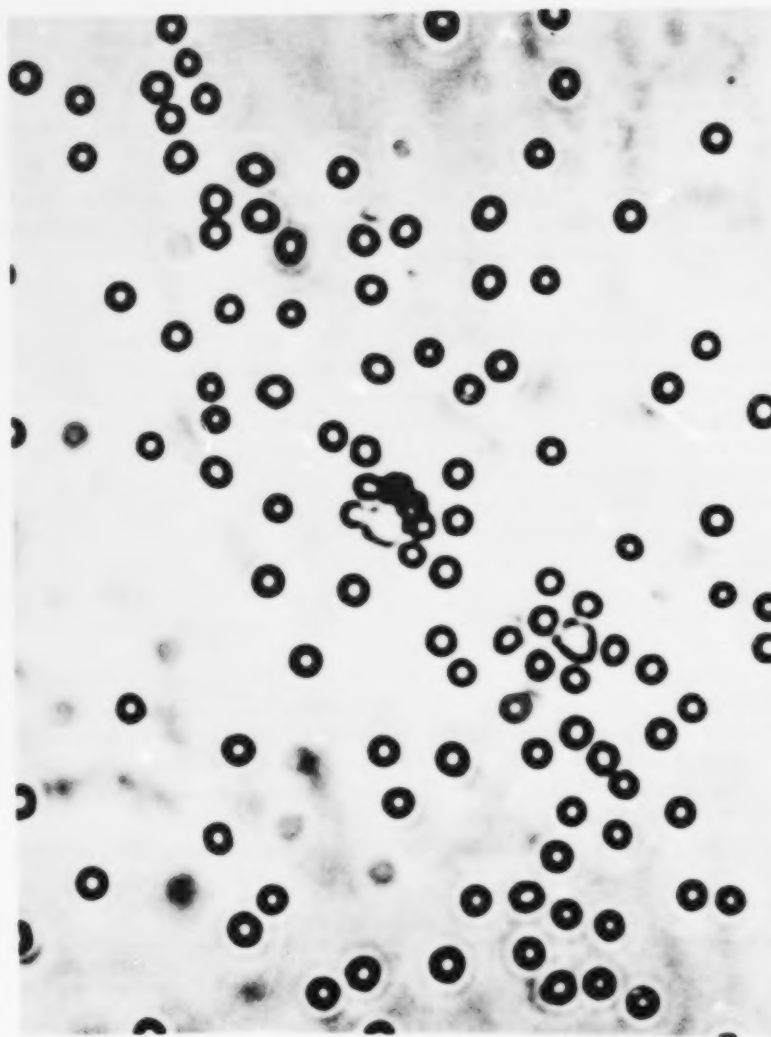


FIG. 1. Rosette formation by a T lymphocyte (center) with sheep red blood cells. $\times 400$.

TABLE 1
MEAN PERCENTAGE OF ROSETTES FORMED BEFORE AND AFTER OZONE
EXPOSURE BY EXPOSURE TIME AND TYPE OF ROSETTE

Exposure time	E Rosette (n = 6)	HEAC Rosette (n = 8)
Pre-O ₃	39.9 (7.6) ^a	19.3 (5.6)
Post-O ₃	33.6 (8.0)	11.9 (6.8)
72 hours post-O ₃	32.9 (9.8)	16.7 (5.2)
2 weeks post-O ₃	40.5 (8.9)	14.0 (8.1)

^a Number within parentheses is SD.

in blood samples collected 72 hours and 2 weeks later. The difference in sample means in pre- and post-ozone exposures, however, was statistically significant only immediately following the exposure ($P = 0.032$).

DISCUSSION

The alteration in B-cell receptors, but not those in T cells, following ozone exposure could be due to the inability of B cells to recognize the HEAC complex since these cells possess receptor sites for C3 and the Fc portion of IgG whereas most T cells do not (Boggs and Winkelstein, 1975). The B-cell membrane may also have been altered by free radical formation (Goldstein and Balchum, 1967), by metabolic disruptions as seen with RBC (Buckley *et al.*, 1975), or by lung cells (Chow and Tappel, 1972, 1973). The alteration of receptors and the cell membrane may occur simultaneously which might decrease or eliminate rosette-forming capabilities. Partial repair of the B cells appeared to occur in these subjects. Maintenance of cell integrity and repair are possible after oxidative stress if this process has not continued to the stage where metabolic mechanisms are destroyed (Wintrobe *et al.*, 1974).

The percentage of RFC found with these subjects corresponds to the normal values obtained by other investigators (Mendes *et al.*, 1973) using the same indicator systems. There was considerable variability in the rosette determination (standard error with T cells of 7.4 % and 3.6% with B cells) which may be inherent in the technique or in the phenomenon being observed. The significance of this variability might be elucidated by the use of skin tests.

The response of the subjects used in this investigation may have been amplified

TABLE 2
ANALYSES OF VARIANCE FOR E AND HEAC ROSETTE DATA^a

Source	df	E Rosette		HEAC Rosette	
		F	P	F	P
Pre-O ₃ vs post-O ₃	1	2.07	0.171	5.30	0.032
Pre-O ₃ vs 72 hours post-O ₃	1	2.49	0.135	0.63	0.432
Pre-O ₃ vs 2 weeks post-O ₃	1	0.02	0.889	2.68	0.117
Error (df: mean square)		(15:58.5)		(21:41.2)	

^a One-factor analysis of variance for repeated measures.

since they had not previously been challenged at this ozone concentration. The ambient air in North Carolina is considerably below this level (Environmental Protection Agency, 1974). These subjects were in good health and were exposed to a single acute dose whereas more sensitive portions of the population or those exposed to diurnal variations may have experienced a different response.

Additional studies are needed to determine the B-cell rosette response to other ozone concentrations, exposure patterns, and recovery times.

CONCLUSION

The ability of B cells to form rosettes with human erythrocytes, sensitized with antiserum and complement, was affected by ozone exposure. A decrease in the mean number of HEAC rosettes formed after a 4-hour exposure to O_3 (0.4 ppm) was observed. This response persisted for at least 2 weeks after exposure but not at the same magnitude as that seen immediately after the challenge. The precise relationship of O_3 exposure to cellular and/or humoral immunities in humans requires further investigation.

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